STUDIES ON THE "GIANT" MUTANT OF *DROSOPHILA MELANOGASTER*

BY

EMMET EDWIN SHIPMAN

A. B., Western Reserve University, 1933

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1940

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STUDIES ON THE "GIANT" MUTANT OF *DROSOPHILA MELANOGASTER*

I. Introduction

Przibram and Megusar,[1] in studying the growth of *Sphodromantis bioculata* (Egyptian Praying Mantis), found that with each ensuing molt, the cast-off integument exhibited a weight double that of the preceding integument; that the weight of the newly molted mantes exhibited a doubling in weight from molt to molt; that the increase in length of the prothorax followed from one molt to another on the average as the cube root of 2 (1.26), while the increase in surface (area) followed from one molt to another as the square root of 2 (1.41), Przibram and Megusar stated that the biological stages follow a geometric progression whose exponent, corresponding to the division of cells into two, is 2. In Sphodromantis, therefore, the increase in size at each molt was said by these authors to be directly due to a simultaneous division of all the cells of the body. They pointed out that the "division steps" might be delayed so that in some instars one would find a quadrupling instead of a doubling, or in other instars, no doubling.

In Drosophila, the body shows a continuous growth, since the integument is only lightly chitinized, and expansion can take place. Alpatov,[2] however, found that the mouth armature in Drosophila does not show a continuous growth, as the rest of the larval body does, but rather shows a discontinuous growth, i.e., the mouth armature grows only at the time of molting. Using the length of the mouth armature of the second instar larva as his basis, Alpatov calculated the length of the first instar and the third instar mouth armatures and found a very close agreement with the measured lengths. He had to multiply the length of the second instar mouth armature twice by the cube-root of 2 to calculate the length at the third instar and divide twice by the cube-root of 2 to calculate the length at the first instar, in order to get this close agreement. He therefore concluded that " . . . the need for a double division and multiplication by 1.26 indicates that each of the moltings in Drosophila larvae is connected with two cell divisions. It is very likely

that the limited number (two) of moltings during the larval life of Diptera cyclorrhapha is a process of secondary reduction, and that in an earlier phylogenetic stage the larvae were characterized by four moltings, each of them linked with one cell division."

Trager,[3] working with the flesh fly, (*Lucilia sericata* Mg.), found that " . . . larval tissues destined to undergo complete histolysis during the pupal stage tend to grow by increase in size of their cells, while tissues which persist to the imago tend to grow by cell multiplication." The tissues which are largely responsible for the bulk of the larval body were found to grow by increase in cell size rather than by increase in cell number. The only tissues studied which increased their bulk by increase in cell number were nervous and blood tissues. The increase in the size of the cells of hypodermis, fat, salivary gland, midgut, hindgut, muscle, and tracheal matrix cells was sufficient to account entirely for the increase in the size of the body of the larva.

This work of Trager's, in combination with other observations reported in the literature, led directly to the conclusion that Alpatov's suggested cell division in Drosphila larvae should be investigated by histological methods.

By a study of the increase in body measurements and increase in size of cells of "bulky" tissues, one should be able to determine whether the increase in size of body was due to (1) increase in the number of cells, (2) increase in the size of each cell, or (3) a combination of increase in cell number and increase in cell size.

II. Methods

Larvae of a gt bb^{11} stock were used for this study. The "giant" mutant stock is one having two phenotypic expressions in the adult: (1) normal sized adults, and (2) giant sized adults. The latter group have a larval developmental period which is on the average four days longer than that of the normal sized adults; and they have a body weight that is on the average 1.7 times that of the normal sized adults. Larvae were raised at 27°C. and collected and fixed every 12 hours throughout their developmental period. The larvae were dehydrated by the normal

butyl alcohol technic. Body measurements were taken, and then a few larvae were selected at random from each collection group and sectioned and stained. Measurements of cell length and nucleus length, width, or diameter were then taken for ventriculus epithelium cells, hindgut epithelium cells, fat body cells, and salivary gland cells. The ventriculus, hindgut, fat body, and salivary gland are all "bulky" tissues and account for at least a large part of the total volume of the larva.

III. Results and Conclusions

The first molt was found to occur at about 40 hours, and the second molt at about 60 hours. Phenotypic non-giant larvae formed the puparium at about 100-128 hours; while phenotypic giant larvae formed the puparium at about 164-212 hours. The first phenotypic non-giant adults appeared at 188 hours; the first phenotypic giant adults at 260 hours.

Changes which occurred in the salivary gland were found to offer a possible method, although not a simple one, for distinguishing between phenotypic non-giant and phenotypic giant larvae. Further study is needed before the method can be offered as more than suggestive. No differences were noticed between the histological structure of wild type larvae described by other authors and the histological structure of larvae in the "giant" mutant strain.

The length of the larval body was found to increase during each instar, as well as at the time of molting. The dorsal ventral dimension, a depth of body measurement, was also found to increase during each instar, as well as at the time of molting. Mouth armature length was found to increase only at the time of molting; that is, mouth armature length was constant during an instar. No significant increase in any of the body measurements was found to occur after 116 hours, although some larvae had not formed the puparium even at 260 hours of development.

Ventriculus epithelium cells and hindgut epithelium cells were found to increase in length at about the same rate and to reach about the same average length. Salivary gland cells were small in the young larvae, but increased in length rapidly during the third instar and were very long in the older third instar

larvae. Fat body cells were longer than ventriculus and hindgut cells throughout the developmental period; and they were longer than the salivary gland cells in young larvae, but about the same average length in the older larvae.

Nuclei of the ventriculus, hindgut, fat body, and salivary gland all contained the salivary gland or Balbiani type of chromosomes. Mitotic figures were never found in these cells.

Simple product-moment coefficients of correlation were determined between various measurements. The coefficient of correlation between body length and dorsal ventral dimension was +0.96, showing that as the larva increased in length it also increased in depth. The coefficient of correlation between body length and length of ventriculus cells was +0.98; between body length and length of hindgut cells, +0.97; between body length and length of fat body cells, +0.97; and between body length and length of salivary gland cells, +0.91. The high, positive correlations between body length and cell length indicated that as the larval body increased in length there was a corresponding and proportional increase in the length of the cells of these "bulky" tissues. This fact, combined with the fact that no mitotic figures were found in any of these cells, led to the conclusion that these tissues contributed to the increase of the larval body only by an increase in the size of their cells, and not by an increase in the number of their cells.

The coefficient of correlation between ventriculus cell length and ventriculus nucleus width was +0.93;between hindgut cell length and hindgut nucleus length, +0.98; between fat body cell length and fat body nucleus diameter, +0.91; and between salivary gland cell length and salivary gland nucleus diameter, +0.97. These high, positive correlations were interpreted as evidence of a proportional increase in size between cell length and nucleus width, length, or diameter.

Photomicrographs of typical cells of the ventriculus, hindgut, fat body, and salivary gland for each of the three instars were included.

No evidence was found to support the idea that phenotypic giant larvae undergo an additional (third) molt and an additional (fourth) instar during the period of extended larval develop-

ment. It was suggested that perhaps the larger body size of the phenotypic giant adult might be due to the fact that during the extended larval developmental period the cells of the imaginal discs, which after all will form most of the organs of the adult, have an additional length of time during which cell proliferation may occur. Suggestions were given for testing this hypothesis in both the larva and adult.

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[2] Alpatov, W. W., 1929. Growth and variation of the larvae of *Drosophila melanogaster*. *J. Exp. Zool.* 52: 407-437.

[3] Trager, W., 1935. The relation of cell size to growth in insect larvae. *J. Exp. Zool.* 71: 489-508.

VITA

Emmet Edwin Shipman was born February 28, 1905, at Akron, Ohio. He received his education in the public schools of Akron, Ohio, and East Cleveland, Ohio, graduating from Shaw High School, East Cleveland, Ohio, in 1924. He attended the Cleveland Institute of Music and studied music under private teachers for the following four years. He attended Battle Creek College from 1928 to 1930, and Adelbert College, Western Reserve University, from 1930 to 1933, receiving his A. B. degree in June, 1933. He attended the Graduate School, Western Reserve University, from February to June, 1933. From 1931 to 1933 he was undergraduate assistant in Biology at Western Reserve University. He entered the Graduate School of the University of Illinois in September, 1933. He held a position as graduate assistant in Zoology at the University of Illinois from 1933 to 1939. He was married to Elsa Johanna Bernds on October 7, 1939.

He is a member of Phi Sigma, Sigma Xi, the Biological Photographic Association, and the Genetics Society of America.